Original Article

Phytochemical and Larvicidal Activity of Pineapple (Annanas comusus L.) Peel Extract Against Aedes aegypti of Dengue Vector

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Abstract

Control of the Aedes aegypti DHF vector with synthetic insecticides has an impact on resistance. As an alternative is the use of plant materials, one of which is pineapple skin waste which is widely available and has not been utilized. This study aims to determine the phytochemical compounds contained and biolarvicidal activity of pineapple peel against Aedes aegypti. Held. Laboratory experimental study with randomized block design effect of concentration on mortality of Aedes aegypti larvae Based on the research results, the ethanol extract of pineapple peel contains flavonoids, tannins, saponins, alkaloids and terpenoids and these compounds have biolarvicidal activity. Ethanol extract at concentrations of 400 ppm and 500 ppm and at concentrations of 100 to 300 ppm has very low activity. Letal Konsentrasi ektrak ethanol kulit nanas adalah Pineapple peel ethanol extract has potential in the future as an environmentally friendly biolarvicidal.

Keywords: Pineapple Pell , Biolarvasida, Aedes aegypti.

INTRODUCTION

Dengue hemorrhagic fever is a problem in many regions of the world, especially in tropical areas, including Indonesia. Palembang City is located in the Province of South Sumatra Indonesia, is one of the endemic cities for dengue fever in Indonesia. Dengue Hemorrhagic Fever (DHF) In 2021 the cumulative DHF in Indonesia was recorded at 71,044 cases with a cumulative number of 690 deaths [1]. Dengue fever is an arbovirus disease yang carried by the Aedes aegypti mosquito is the primary vector in the transmission of DHF [2], [3].

The people and government of Indonesia have tried to overcome dengue fever by controlling vectors because there is no DHF vaccine yet. Control is carried out in various ways, but the most preferred and widely practiced method is chemical control using insecticides.

The use of insecticides for a long time and with improper management can have a negative impact on the environment and dengue hemorrhagic fever vectors, which can cause resistance to Aedes aegypti dengue hemorrhagic vectors.. The results of molecular research on Aedes aegypti in Palembang found mutations in the VGSC gene at the Val1016Ile point, as proof of the resistance mechanism which is a target site for synthetic pyrethroid insecticides [4].

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There is a need for efforts to control the dengue vector using environmentally friendly insecticides. One of the plants that is widely available in the city of Palembang is the Queen variety pineapple plant which has been adapted for a long time in the province of South Sumatra, known as the Palembang pineapple. Pineapple produces solid waste in the form of pell and weevil. Pineapple waste can reach 48.6% of the total weight of the fruit, consisting of pineapple pell and hump [5]. Pineapple pell is an untapped waste, even though pineapple pell contains vitamin C, carotenoids and flavonoids [17].

Pineapple skin is thought to contain phytochemicals that act as insecticides, anti-bacterial and anti-fungal. Pineapple peel from Rayalseema region of Andhra Pradesh, India contains phytochemical compounds, namely flavonoids, tannins, saponins, sterols, terpenoids and alkaloids, these compounds can be used as raw materials for insecticides According to [7, 8], Ethanol extract of pineapple fruit contains saponins, tannins, flavonoids and terpenoids that act to inhibit the growth of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus amyloliquefacien bacteria with an inhibition zone of 15-17 mm. Pineapple pell waste originating from fruit traders contains flavonoids, alkaloids, anthocyanins and tannins and pineapple peel has a high inhibitory power against the activity of E. coli and S. Aurens bacteria. Pineapple peel extract at a concentration of 4% was able to kill Culex mosquitoes up to 97.5% [9- 10-11-12].

This study aims to determine the phytochemical content of the ethanol extract of pineapple peel which acts as a biolarvicidal against Aedes aegypti as a vector of dengue hemorrhagic fever.

MATERIALS AND METHODS

Study Area

This study is a laboratory experimental study with a randomized block design. To test the concentration of pineapple peel ethanol extract on larval mortality and determine the phytochemical content. The research was carried out in the Special Integrated Laboratory of the Sriwijaya University Palembang Postgraduate Laboratory and Baturaja Health Research and Development Center Laboratory. It will be held from March to June 2022.

The materials used in this study are pineapple peel used in this study was collected from pineapple planting centers, East Prabumulih District, Prabumulih City, South Sumatra Province Indonesia Latitude south side 3°-4°, Longitude Eartern104°-105°, Altitude 48 m, Annual Raining Average 2.723 mm/year, Daili Temperatur average 20.13 o C, Comperative humadity average 79,03% and type of soil alluvial, clay and sandy [13]. Egg Aedes aegypti Strain Liver Pool from Health Research and Development entomology laboratory. While the chemicals used in this study were aluminum foil, filter paper, parchment paper, ethanol p.a (Merck™), distilled water, AlCl3 (Merck™), and potassium acetate (Merck™), HCl 2 N, Mg, FeCl2 , reaction Meyer, Wagner, Dragendrof. Chloroform, H2So4, Lieberman Burchar reagent.The tools used are, blender(Philips™), ayakan no.60 dan 40, rotary evaporator (IKA™ HB 10), vacum pump (B-One™), waterbath (MEMMERT™), perangkat alat gelas, mikro pipet, cawan porselen, inkubator/dry box (PH050 A™), dan timbangan analitik (BEL™).

Preparasi Sample

Pineapple pell that has been cleaned cut into small pieces and then dried in the sun not directly covered with a black cloth covered. Then weigh the dry weight. As much as 500 g was crushed using a blender and sieved with a size of 40 – 60 mesh and crushed to obtain simplicia powder. then the pineapple skin simplicia was extracted using 2 L ethanol pa 96% as a solvent by the maceration method. Furthermore, it was evaporated using a rotary evaporator at 60 oC to obtain a thick extract

Phytochemical Test.

Flavonoid Test. 2 ml of the solution was put into a test tube, then heated for 15 minutes, after which 1 ml of concentrated HCl and 0.05 mg of Mg powder were added. If a red, yellow or orange color is formed, this indicates the presence of flavonoids [10]. Tannin test. A total of 1 ml of the solution was put into a test tube, then 2-3 drops of 1% FeCl3 solution were added. If a dark blue or blackish green color is formed, it indicates the presence of tannins [14]. Saponin Test. 2 ml of the test solution is put into a test tube, then added with 10 ml of hot water, after that it is cooled and shaken for 10 seconds, if there is foam about 10 minutes with a height of 1 to 10 cm and when 2N HCl is added, the foam does not disappear, indicating the presence of saponins [15] .Alkaloid Test. 0.5 grams of thick pineapple peel extract was weighed, dissolved with 1 ml of 2N HCl and 9 ml of aquadest, then put into a test tube and then heated on a water bath for 2 minutes, then cooled and filtered. The resulting filtrate was then divided into 3 test tubes where each of the Mayer, Wagner and Dragendorf reagents was added. If a white precipitate is formed in Mayer's reagent, an orange precipitate in Dragendorf's reagent and a brown precipitate in Wagner's reagent, indicates the presence of alkaloids [16].Terpenoid Test. The test extract was dissolved in chloroform and then filtered. The filtrate is then added with a few drops of concentrated sulfate. If a brownish red color is formed at the boundary between the two phases, this indicates that terpenoids are present [17].

Bioassay Larvasida

Eggs of Aedes aegypti Strain liverpool F 146 were maintained until instar. Each treatment consisted of five replications.A total of 100 ml of pineapple peel extract from its concentrations of 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm were put into a 250 ml disposable plastic cup, 25 Aedes aegypti larvae were added. Observations were made on dead larvae for 24 hours. Observation of the percentage of larval mortality is carried out by

$$\% \text{ mortality of tested larvae} = \frac{\sum \text{dead tested larvae}}{\sum \text{tested larvae}} \times 100$$

Data analysis

The larval mortality percentage data were tested for normality and homogeneity. If the data is normally distributed and homogeneous, then it is continued with Analysis of Variance (ANOVA), if the ANOVA test results show a significant effect, then it is continued with the Tukey test. To get the value of LD 50 is done by probit test.

RESULTS

Results of the phytochemical test of the Palembang pineapple pell contained phytochemical compounds flavonoids, tannins, saponins, alkaloids, terpenoids, but did not contain steroids contain steroids.

Table Pineapple Pell Phytochemical Compounds Derived from 5 production center in South Sumatra.

Phytochemical compounds	content	Reagent	Color indicators
Flavanoid	+++	HCl	Black, Reddish
Tanin	++	FeCl3 10%	Dark blue
Saponin	+++	Aquadest	Stable foam
		+ HCl	
Alkaloid	+++	Dragendorff	Orange red precipitate
Alkaloid	+++	Wagner	Chocolate Deposit
Alkaloid	+++	Mayer	White precipitate
Terpenoid	++	Lieberman Buercard	brown ring

Note:: +++ (strong)

++ (medium)

+ (weak)

The results of the data normality test showed that the data was normally distributed with p 0.05 = 0.725. 0.05 and the results of the homogeneity test with p 0.05 = .007 showed homogeneous data, then continued with analysis of variance (ANOVA) to see the effect of the concentration of ethanol extract as a biolarvicide on the death of Aedes aegtpti. Based on ANOVA, it showed that the concentration of ethanol extract had a significant effect with an F-value of 130.837 greater than F-table and P0.05 = 0.000 <0.05, indicating that biolaevasida had a significant effect on the death of Aedes aegypti. The results of the ANOVA test can be seen in the following table 1.

Table 1. ANOVA Effect of pineapple peel ethanol extract concentration on the death of Aedss aegypti.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	776,300	4	194,075	130,837	0,000
Within Groups	22,250	15	1,483		
Total	798,550	19			

Based on the results of the ANOVA test, Tukey's follow-up test was carried out to see the differences between the concentrations of the pineapple peel ethanol extract, the results of the Tukey test are presented in the following table 2.

The results of the Tukey test showed a significant difference for all concentrations, except between 400 pp and 500 ppm, there was no significant difference in the death of Aedes aegypti larvae.

Table 2. Effect of concentration of ethanol extract of pineapple peel on the mortality of Aedes aegypti larvae

Concentration (ppm)	Larvvae Mortality	Percentage of Larval Mortality
100	7,75 (4,22 - 11,28) a	31 (16.88 - 45.12) a
200	17,25 (15,73 - 18,77) b	69 (62.92 - 72.08) b
300	20,00 (18,70 - 21,30) c	80 (74.80 - 85.20) c
400	24,25 (22,73 - 25,00) d	97 (90.92 - 100.0) d
500	25,00 (25,00 -25,00) d	100 (100.0 - 100.0) d

Values represent the mean ±standard deviation (SD), n=5

Values in the same column with different superscript letters are significantly different Tukey test ($p < 0.005$)

The effect of the concentration of the ethanol extract of pineapple peel at a concentration of 100 ppm showed a low action as an Aedes aegypti biolarvicidal, but with an increase in concentration the biolarvicidal activity of the extract increased and at a concentration of 500 ppm it could kill 100 Aedes aegypti larvae (Figure 1).

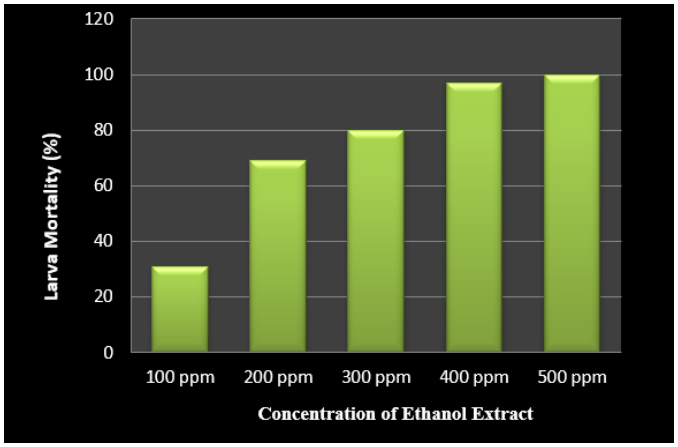

Figure 1. Effect of Ethanol Extract Concentration on Total Larval Mortality

The results of the probit analysis Lethal Concentration 50 (LC 50) of the ethanol extract of pineapple peel was 144.04 ppm and the maximum killing power was 408.93 ppm (LC 95). The correlation between extract concentration and activated larvicide is very close with a value of r = 0.94 and the regression equation can be seen in the table 3.

Table 3. Lethal Concentration of Pineapple Peel Ethanol Extract Against Aedes aegypti Larvae.

LC 50 (ppm)	LC 95 (ppm)	r	Persamaan regresi
144,04 (89.70-187.19)	408,93(297,91-856,31)	0.94	Y = 12.5 + 0.175 x

Discussion

Pineapple peel extract has the potential as a biolarvicidal based on the results of analysis containing phytochemical compounds of flavonoids, saponins, tannins, alkaloids and terpenoids.Hasil penelitian yang dilakukan oleh [18]., ektrak etanol kulit nanas mengandung senyawa fitokimia flavonoid, tanin, saponin dan alkaloid. The content of different secondary metabolites in a plant will cause a distinctive insecticidal effect on insects [18]. The toxicity of pineapple peel to Aedes aegypti larvae is due to the phytochemical compounds it contains, where flavonoids can inhibit breathing and cause nerve wilting, saponins can reduce mucous membranes, alkaloids can act as stomach poisons, tannins can damage tissues and terpenoids can damage breathing [18, 19].

Concentrations of 100 ppm to 300 ppm larvicidal activity of the ethanol extract of pineapple peels are low, but with increasing concentrations of 400 ppm and 500 ppm the activity of the ethanol extract of pineapple peels is very goodhigher concentrations disrupt the respiration and stomach of the larvae. The larval stage represents the most delicate stage in the life cycle of insect vectors. Therefore, efforts to control these vectors are usually directed at the larval stage for disrupting their biological cycle by different strategies [20].

The lethal concentration (LC 50) of the ethanol extract of pineapple peel was 144.04 ppm and the maximum killing power of LC 95 was 408.93 ppm. This shows that the ethanol extract of pineapple peel is toxic to Aedes aegypti larvae. LC50 1000 mg/L = Toxic and LC 50 500 ppm and is said to be active [21]

Conclusion

Pineapple peel extract has the potential to be developed as an environmentally friendly Aedes aegypti biolarvicidal that can replace synthetic insecticides in controlling Aedes aegypti as a vector of dengue hemorrhagic fever in its larval stage. Concentrations up to 300 ppm very low activity and 500 ppm concentration 100 percent mortality of Aedes aegypti larvae.

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